

**AMENDMENTS TO THE CLAIMS:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**LISTING OF CLAIMS:**

1. (Withdrawn) A purified preparation containing a polynucleic acid encoding at least one polypeptide selected from the group consisting of:
  - proteins encoded by one or more open reading frames (ORF's) of an Iowa strain of porcine reproductive and respiratory syndrome virus (PRRSV);
  - proteins at least 80% but less than 100% homologous with those encoded by one or more of ORF 2, ORF 3, ORF 4 and ORF 5 of an Iowa strain of PRRSV;
  - proteins at least 97% but less than 100% homologous with proteins encoded by one or both of ORF 6 and ORF 7 of an Iowa strain of PRRSV; and
  - antigenic regions of said proteins which are at least 5 amino acids in length and which effectively stimulate immunological protection in a porcine host against a subsequent challenge with a PRRSV isolate;
  - and combinations thereof.

2. (Withdrawn) The purified preparation of Claim 1, wherein said polynucleic acid has a sequence selected from the group consisting of the formulas (I), (II) and (III):

5'-α-β-γ-3'	(I)
5'-γ-δ-ε-3'	(II)
5'-α-β-γ-δ-ε-3'	(III)

wherein:

α encodes at least one polypeptide, or antigenic fragment thereof having a length of at least 5 amino acid residues, encoded by a polynucleotide selected from the group consisting of ORF 1a and 1b, ORF 2 and ORF 3 of a PRRSV and regions thereof encoding the antigenic fragments;

β is either a covalent bond or a linking polynucleic acid which excludes a sufficiently long portion of ORF 4 from an hv PRRSV to render the hv PRRSV either low-virulent or non-virulent;

γ is at least one copy of an ORF 5 from an Iowa strain of PRRSV;

δ is either a covalent bond or a linking polynucleic acid which does not materially affect transcription and/or translation of said polynucleic acid; and

$\epsilon$  encodes at least one polypeptide encoded by either a polynucleotide selected from the group consisting of ORF 6 and ORF 7 of an Iowa strain of PRRSV, or a region of ORF 5, ORF 6 and ORF 7 of an Iowa strain of PRRSV encoding an antigenic polypeptide fragment having a length of at least 5 amino acid residues;

and when  $\delta$  is a covalent bond,  $\gamma$  may have a 3'-end which excludes the region overlapping with the 5'-end of a corresponding ORF 6.

3. (Withdrawn) The purified preparation of Claim 1, wherein said ORF 5 is from a high replication (hr) phenotype.

4. (Withdrawn) The purified preparation of Claim 1, wherein  $\epsilon$  is a polynucleotide encoding an antigenic region of ORF 6.

5. (Withdrawn) The purified preparation of Claim 1, wherein said polypeptide is selected from the group consisting of proteins at least 97% homologous with those encoded by ORF's 6-7 of VR 2385, VR 2429 (ISU-22), ISU-79 and VR 2431 (ISU-3927); proteins at least 90% homologous with proteins encoded by ORF's 2-5 of VR 2385, VR 2429, VR 2430 (ISU-55), VR 2431, ISU-79 and ISU-1894; and antigenic regions of said proteins having a binding affinity of at least 1% of the binding affinity of the full-length protein encoded by the corresponding ORF 2, 3, 4 or 5 of VR 2385, VR 2429, ISU-79 or VR 2431 or ORF 6 or 7 of VR 2385, VR 2429, VR 2430, VR 2431, ISU-79 or ISU-1894 to a monoclonal antibody which specifically binds to said full-length protein; and combinations thereof.

6. (Withdrawn) The purified preparation of Claim 5, wherein isolated polynucleic acid is selected from the group consisting of ORF 2, ORF 3, ORF 4, ORF 5, ORF 6 and ORF 7 of any one of VR 2385, VR 2429, VR 2431, ISU-79, ISU-3927, ISU-22 and ISU-1894, and combinations thereof.

7. (Withdrawn) The purified preparation of Claim 5, wherein said polypeptide is encoded by at least one of ORF's 2, 3, 5, and 6 of VR 2385, VR 2429, VR 2431, ISU-79, ISU-22 and ISU-1894.

8. (Withdrawn) The purified preparation of Claim 1, wherein said polynucleic acid encodes said homologous protein, and non-homologous residues in said homologous protein are conservatively substituted.

9. (Withdrawn) The purified preparation of Claim 1, wherein said isolated polynucleic acid encodes said antigenic region of at least one of said proteins, said antigenic region having a length of from 5 amino acids to less than the full length of said protein.
10. (Withdrawn) The purified preparation of Claim 9, wherein said antigenic region has a binding affinity to a monoclonal antibody which specifically binds to said protein of at least 1% of the binding affinity of said protein to said monoclonal antibody.
11. (Withdrawn) A purified polypeptide encoded by the polynucleic acid of Claim 1 or 2.
12. (Withdrawn) A purified polypeptide encoded by the polynucleic acid of Claim 5 or 6.
13. (Withdrawn) A vaccine, comprising an effective amount of the polypeptide of Claim 11 to raise an immunological response in a pig against a porcine reproductive and respiratory syndrome virus, and a physiologically acceptable carrier.
14. (Withdrawn) A vaccine, comprising an effective amount of the polynucleic acid of Claim 1 or 2 to raise an immunological response in a pig against a porcine reproductive and respiratory syndrome virus, and a physiologically acceptable carrier.
15. (Withdrawn) The vaccine of Claim 13, wherein said virus causes a disease characterized by one or more of the following symptoms and clinical signs: respiratory distress, fever, and a reproductive condition in a sow selected from the group consisting of abortion, stillbirth, weak-born piglets, type II pneumocyte formation, myocarditis, encephalitis, alveolar exudate formation and syncytia formation.
16. (Withdrawn) The vaccine of Claim 14, wherein said virus causes a disease characterized by one or more of the following symptoms and clinical signs: respiratory distress, fever, and a reproductive condition in a sow selected from the group consisting of abortion, stillbirth, weak-born piglets, type II pneumocyte formation, myocarditis, encephalitis, alveolar exudate formation and syncytia formation.
17. (Withdrawn) A method of protecting a pig from infection by a porcine reproductive and respiratory syndrome virus, comprising administering an effective amount of the vaccine of Claim 13 to a pig in need thereof.

18. (Withdrawn) The method of Claim 17, wherein said vaccine is administered orally or parenterally.

19. (Withdrawn) The method of Claim 18, wherein said vaccine is administered intramuscularly, intradermally, intravenously, intraperitoneally, subcutaneously or intranasally.

20. (Withdrawn) The method of Claim 17, wherein said vaccine is administered to a sow in need thereof.

21. (Withdrawn) An antibody which specifically binds to the polypeptide of Claim 11.

22. (Withdrawn) The antibody of Claim 21, wherein said antibody is a monoclonal antibody.

23. (Withdrawn) An antibody which specifically binds to the polypeptide of Claim 12.

24. (Withdrawn) A method of treating a pig suffering from porcine reproductive and respiratory syndrome, comprising administering an effective amount of the antibody of Claim 21 to a pig in need thereof.

25. (Withdrawn) A diagnostic kit for assaying a porcine reproductive and respiratory syndrome virus, comprising the antibody of Claim 21 and a diagnostic agent which indicates a positive immunological reaction with said antibody.

26. (Withdrawn) The diagnostic kit of Claim 25, wherein said antibody is a biotinylated monoclonal antibody, said diagnostic agent comprises peroxidase-conjugated streptavidin and a peroxidase.

27. (Withdrawn) The diagnostic kit of Claim 26, further comprising aqueous hydrogen peroxide, a protease which digests the porcine tissue sample, a fluorescent dye and a tissue stain.

28. (Withdrawn) A method of diagnosing infection of a pig by or exposure of a pig herd to a porcine reproductive and respiratory syndrome virus, comprising the steps of: incubating ascites fluid comprising the monoclonal antibody of Claim 22 with a tissue sample for a sufficient length of time and at an appropriate temperature to provide essentially

complete immunological binding to occur between said monoclonal antibody and one or more viral antigens in said tissue sample;

incubating a biotinylated linking antibody with the monoclonal antibody-treated tissue sample;

incubating a peroxidase-conjugated streptavidin with the biotinylated antibody-treated tissue; and

detecting said viral antigens.

29. (Withdrawn) The method of Claim 28, further comprising, prior to said incubating steps, the sequential steps of removing endogenous peroxidase from an isolated porcine tissue sample with aqueous hydrogen peroxide, and digesting said tissue sample with a sufficient amount of an appropriate protease to expose said viral antigens; and after said second incubating step, the sequential steps of incubating the peroxidase-conjugated streptavidin-treated tissue with a chromagen and a stain, and detecting said viral antigens, wherein observation of stained chromagen-treated tissue is indicative of the presence of said viral antigens.

30. (Currently amended) A diagnostic kit for the differential diagnosis of North American and European porcine reproductive and respiratory syndrome virus (PRRSV), comprising:

(a) a first primer comprising a polynucleotide having a sequence of from 10 to 50 nucleotides in length which selectively hybridizes to a genomic polynucleic acid from a North American strain of PRRSV, wherein said North American strain is an Iowa strain of PRRSV selected from the group consisting of ISU-22 (VR 2385 or VR 2386), ISU-22 (VR 2429), ISU-55 (VR 2430), ISU-3927 (VR 2431), ISU-79 (VR 2474) and ISU-1894 (VR 2475), at a temperature of from 25 to 75°C,

(b) a second primer comprising a polynucleotide having a sequence of from 10 to 50 nucleotides in length which selectively hybridizes to said genomic polynucleic acid from said Iowa strain of PRRSV at a temperature of from 25 to 75°C and being downstream from the sequence to which said first primer hybridizes, and

(c) a reagent which enables detection of an amplified polynucleic acid, wherein said primers selectively amplify said North American strain and not a European PRRSV strain, wherein said European strain is Lelystad.

31. (Original) The diagnostic kit of Claim 30, wherein said reagent is an intercalating dye, the fluorescent properties of which change upon intercalation into double-stranded DNA.

32. (Withdrawn) A method of producing a vaccine which confers immunological protection against a subsequent challenge with a porcine reproductive and respiratory syndrome virus, comprising the steps of infecting a suitable host cell with the polynucleic acid of Claim 1 and culturing said host cell.

33. (Withdrawn) The method of Claim 32, further comprising the step of isolating at least one of said cultured host cell and polypeptide encoded by said polynucleic acid.

34. (Withdrawn) A method of producing the vaccine of Claim 14, comprising the steps of infecting a suitable host cell with at least one of said polynucleic acid and a virus containing said polynucleic acid, culturing said host cell, and isolating said polynucleic acid from said cultured host cell.

35. (Withdrawn) The method of Claim 34, wherein said infecting step employs said virus, and said isolating step comprises:

- (A) collecting a sufficiently large sample of said virus to isolate said polynucleic acid,
- (B) isolating said polynucleic acid from said collected virus, and
- (C) combining said polynucleic acid with said physiologically acceptable carrier.

36. (Withdrawn) The method of Claim 35, wherein said virus or infectious agent is collected from a source selected from the group consisting of a culture medium, cells infected with said virus, and both a culture medium and cells infected with said virus.

37. (Withdrawn) A biologically pure culture of a virus containing the polynucleic acid of Claim 1.

38. (Withdrawn) The biologically pure culture of Claim 37, wherein said polynucleic acid further contains a gene encoding a polypeptide adjuvant or an antigen other than a porcine reproductive and respiratory syndrome virus antigen.

39. (Previously presented) A diagnostic kit for assaying a porcine reproductive and respiratory syndrome virus, comprising:

(a) a first primer comprising a polynucleotide having a sequence of from 10 to 50 nucleotides in length which hybridizes to a genomic polynucleic acid from an Iowa strain of porcine reproductive and respiratory syndrome virus selected from the group consisting of ISU-22 (VR 2385 or VR 2386), ISU-22 (VR 2429), ISU-55 (VR 2430), ISU-3927 (VR 2431), ISU-79 (VR 2474) and ISU-1894 (VR 2475), and also hybridizes to polynucleic acid from Lelystad virus at a temperature of from 25 to 75°C,

(b) a second primer comprising a polynucleotide having a sequence of from 10 to 50 nucleotides in length which hybridizes to said genomic polynucleic acid from said Iowa strain of porcine reproductive and respiratory syndrome virus, and also hybridizes to polynucleic acid from Lelystad virus at a temperature of from 25 to 75°C and being downstream from the sequence to which said first primer hybridizes, and

(c) a reagent which enables detection of an amplified polynucleic acid.

40. (Previously presented) The diagnostic kit of Claim 39, wherein said reagent is an intercalating dye, the fluorescent properties of which change upon intercalation into double-stranded DNA.

41. (New) The diagnostic kit of Claim 39, wherein said primers are selected from the group consisting of SEQ ID NOS:8 and 9, SEQ ID NOS:9 and 10, and SEQ ID NOS:11 and 12.